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Toxicity and carcinogenicity of methyl isobutyl ketone in F344N rats and B6C3F1 mice following two year inhalation exposure

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Abstract

Methyl isobutyl ketone (MIBK) is primarily used as a denaturant for rubbing alcohol, as a solvent and in the manufacture of methyl amyl alcohol. Inhalation of vapors is the most likely route of exposure in the work place. In order to evaluate the potential of MIBK to induce toxic and carcinogenic effects following chronic exposure, groups of 50 male and 50 female F344/N rats and B6C3F1 mice were exposed to MIBK at concentrations of 0, 450, 900, or 1800 ppm by inhalation, 6 hours per day, 5 days per week for two years. Survival was decreased in male rats at 1800 ppm. Body weight gains were decreased in male rats at 900 and 1800 ppm and in female mice at 1800 ppm. The primary targets of MIBK toxicity and carcinogenicity were the kidney in rats and the liver in mice. In male rats, there was increased mineralization of the renal papilla at all exposure concentrations. The incidence of chronic progressive nephropathy (CPN) was increased at 1800 ppm and the severity was increased in all exposed groups. There were also increases in renal tubule hyperplasia at all exposure concentrations, and in adenoma and adenoma or carcinoma (combined) at 1800 ppm; these lesions are thought to represent a continuum in the progression of proliferative lesions in renal tubule epithelium. These increases may have resulted from the increased severity of CPN, either through α2u-globulin dependent or independent mechanisms. An increase in mononuclear cell leukemia at 1800 ppm was an uncertain finding. Adrenal medulla hyperplasia was increased at 1800 ppm, and there was a positive trend for increases in benign or malignant pheochromocytomas (combined). In female rats, there were increases in the incidence of CPN in all exposure concentrations and in the severity at 1800 ppm, indicating that CPN was increased by mechanisms in addition to those related to α2u-globulin. There were renal mesenchymal tumors,

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which have not been observed in historical control animals, in two female rats at 1800 ppm. The relationship of these tumors to exposure to MIBK was uncertain. Hepatocellular adenomas, and adenoma or carcinoma (combined) were increased in male and female mice exposed to 1800 ppm. There were also treatment-related increases in multiple adenomas in both sexes.

Keywords

methyl isobutyl ketone; carcinogenicity; National Toxicology Program; inhalation; kidney; liver

1. Introduction

Methyl isobutyl ketone (MIBK), an important industrial chemical, is classified as a volatile organic compound, with a vapor pressure of 15 mmHg at 20°C. In 1995 and 1996, the United States production of MIBK was 80,000 metric tons (CMA, 1997), and the projected demand for 2006 has been calculated at 147 million pounds (CMR, 2004). Primarily, MIBK is used as a denaturant for rubbing alcohol, as a solvent for paints, varnishes, nitrocellulose, and lacquers and in the manufacture of methyl amyl alcohol (IPCS, 1990). Methyl isobutyl ketone is also used in industrial extraction processes, as a solvent for protective coatings and in rare metals extraction and in dewaxing of mineral oils, in drycleaning preparations and in the synthesis of methyl isobutyl carbinol. The most likely exposures in the work place are primarily by inhalation of the vapors, although exposures by contact with the skin and eyes also occur. Time-weighted average (205 mg/m^3; 50 ppm) and short-term exposure limit (307 mg/m^3; 75 ppm) values have been recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 2005).

MIBK is readily absorbed into the bloodstream after inhalation exposure and is likely to be widely distributed in the body (Duguay and Plaa, 1995). Metabolism occurs by reduction of the carbonyl group to a secondary alcohol, 4-methyl-2-pentanol and oxidation at the ω-1 carbon atom to form a hydroxylated ketone, 4-hydroxymethyl isobutyl ketone, also known as diacetone alcohol (DiVincenzo *et al.*, 1976). 4-Methyl-2-pentanol may be further conjugated with sulphate or glucuronic acid, or may enter intermediary metabolism to be eliminated as CO_2, or may be incorporated into tissues. These metabolites have been measured in the tissues of rats following MIBK exposure (Duguay and Plaa, 1995; Granvil, et al., 1994). Induction of P450 enzyme activities by MIBK (Lapadula *et al*., 1991; Raymond and Plaa 1995a) and potentiation of the toxic effects produced by other chemicals, by MIBK or metabolites of MIBK, have been reported (Abou-Donia, et al., 1985; Plaa and Ayote, 1985; Raymond and Plaa, 1995a; Raymond and Plaa, 1995b; Vezina *et al*., 1990; Vezina and Plaa, 1988).

The toxicity of MIBK in short-term inhalation studies has been characterized. Groups of six male and female F344 rats and B6C3F1 mice were exposed by inhalation to 100, 500, or 2,000 ppm MIBK, 6 hours/day; there were 9 exposures over 11 days (Phillips, et al., 1987). Lethargy and lacrimation were observed in high dose animals, but no ophthalmologic lesions or changes in body weight were found. There were increases in absolute and relative liver and kidney weights in both rats and mice at various exposure concentrations. The primary microscopic findings were hyaline droplet formation (500 or 2,000 ppm) and epithelial regeneration of the proximal convoluted tubule cells (2,000 ppm) in the male rat kidney. In the liver, there were increased mitotic figures (qualitative assessment) in 1 female rat and 2 male rats, hepatic mitosis in 1 female mouse, and glycogen depletion in 4 female mice.

Groups of 14 male and female F344 rats and B6C3F1 mice were exposed by inhalation to 50, 250, or 1,000 ppm methyl isobutyl ketone for 14 weeks (Phillips *et al.*, 1987). One male mouse exposed to 1,000 ppm died near the end of the study of an unknown cause. Terminal body

weights of dosed rats and mice were similar to controls, except for slight but significant increases in females at 250 and 1,000 ppm. Changes in organ weights included increases in absolute liver weight (male rats at 50 or 1,000 ppm, male mice at 250 or 1,000 ppm), relative liver weight (male mice at 1,000 ppm), and absolute kidney weight (female rats at 250 ppm). There were increases in serum cholesterol levels in male rats exposed to 250 or 1,000 ppm, in urinary glucose excretion in male rats exposed to 250 ppm and in male and female rats exposed to 1,000 ppm, and in urinary total protein excretion in male rats exposed to 1,000 ppm. There was an increase in both the incidences and extent of hyaline droplets in the kidneys of male rats exposed to 250 or 1,000 ppm. There were no changes in serum or urine biomarkers of injury or in histopathology in mice.

Male and female 7 week old Sprague-Dawley rats were exposed to 0, 500, 1,000 or 2,000 by inhalation for at least 70 days as part of a two-generation reproductive study (Nemec, et al., 2004). There were increases in both absolute and relative kidney weights in males. Histologic changes suggestive of CPN were clearly present in Sprague-Dawley rats exposed to 1,000 or 2,000 ppm MIBK. There were also increases in absolute and relative liver weights of male and female rats exposed to 2000 ppm and associated exposure-related centrilobular hepatocellular hypertrophy in males exposed to 500, 1,000, or 2,000 ppm. The adrenal weights of F0 females were increased at 2,000 ppm.

The available data (O'Donoghue *et al.*, 1988; Zeiger *et al*., 1992) suggest that MIBK is not genotoxic. To our knowledge, there are no other published reports of the carcinogenic potential of MIBK in animal models or epidemiologic studies in humans.

The objective of present studies was to characterize the toxicity and carcinogenicity of MIBK in F344 rats and B6C3F1 mice following inhalation exposure to 0, 450, 900 or 1800 ppm for 2-years.

2. Materials and Methods

2.1. Test article

MIBK was obtained from ChemCentral (Kent, WA) in one lot. The overall purity was determined to be greater than 99%. The bulk chemical was stored at room temperature and no degradation was detected.

2.2. Inhalation exposure system

Inhalation exposures were conducted at Battelle Toxicology Northwest Operations (Richland, WA). MIBK was pumped onto the heated surface of the study laboratory-designed wick generator, where it was vaporized. The MIBK concentrations in the exposure chambers were monitored by an on-line gas chromatograph approximately every 28 minutes. Buildup and decay rates for chamber vapor concentrations were determined with animals present in the chambers and the time to achieve 90% of the target concentration after the beginning of vapor generation (T90) was 12 minutes. Evaluations of chamber uniformity and persistence and monitoring for MIBK degradation impurities were conducted periodically throughout the studies by gas chromatography. Chamber uniformity was maintained and no degradation was detected.

2.3. Study Design

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in these studies. Rats and mice were housed individually. Chambers, racks, and cages were changed weekly and cages were rotated weekly. Chambers were maintained at a temperature of 75 ± 3°F, a relative humidity of 55% ± 15%, a

twelve hour light-dark cycle, and 15 ± 2 air changes per hour. NTP-2000 non-purified diet (irradiated wafers; obtained from Zeigler Brothers, Inc.) was available *ad libitum* except during exposure periods and changed weekly; tap water was available *ad libitum* via an automatic watering system. Prior to the initiation of the studies, study animals were quarantined for approximately two weeks and were approximately 6 weeks old at the beginning of the studies. The rats and mice were distributed randomly into exposure groups of approximately equal initial mean body weights and identified by tail tattoo. Groups of 50 male and 50 female individually housed rats and mice were exposed to MIBK at concentrations of 0, 450, 900, or 1800 ppm, 6 hours plus T90 per day, 5 days per week for two years. Animals were killed by asphyxiation with CO_2. The average age of rats at necropsy was 110 weeks.

All animals were observed twice daily. Body weights were recorded initially and clinical findings and body weights were recorded weekly for the first 13 weeks, monthly until the last four months of the studies, every 2 weeks thereafter, and at the end of the studies. Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 microns, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. An extended evaluation of the kidney was performed in male rats because the incidence of renal neoplasms suggested a potential carcinogenic effect. For extended evaluation of renal proliferative lesions, the residual kidney tissue was step-sectioned at 1 mm intervals, to obtain 3 to 4 additional sections from each kidney.

Animal use was in accordance with the United States Public Health Service policy on humane care and use of laboratory animals and the Guide for the Care and Use of Laboratory Animals. These studies were conducted in compliance with the Food and Drug Administration Good Laboratory Practice Regulations (21CFR, Part 58).

2.4. Statistical Methods

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided. Survival rates are expressed as percentages, out of 50 animals, unless otherwise noted. Body weight data were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. Unless otherwise specified, a value of k=3 was used in the analysis of site specific lesions. Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall exposure-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided.

3. Results

3.1. Rats

Survival of the 1800 ppm males was significantly less than that of the controls (0 ppm, 32/50; 450 ppm, 28/49; 900 ppm, 25/50; 1800 ppm, 19/50). Mean body weights of 900 ppm male rats were 6–8 % less after week 97 and 1800 ppm males were 5–8 % less after week 89, than those

of the chamber control group. Mean body weights and survival (0 ppm, 35/50; 450 ppm, 34/50; 900 ppm, 26/50; 1800 ppm, 32/50) of all exposed groups of females were similar to that of the controls.

CPN similar to that which occurs in aged rats was observed in males and females in all groups, including controls (Tables 1 and 2). In males, significant increases in incidence were observed at 1800 ppm and in severity at all exposure concentrations. The incidence of mineralization was also significantly increased at all exposure concentrations in males; severity was generally increased in exposed groups (Table 1). In female rats, increased incidences of CPN were significant in all exposed groups. The average severity of CPN ranged from minimal to mild and was increased in exposed females at 1800 ppm.

CPN is an age-related disease process. In both sexes, changes consisted of a spectrum of lesions that included varying degrees of renal tubule dilatation with and without hyaline (proteinaceous) casts, multifocal degeneration, regeneration, and hypertrophy of the tubular epithelium; thickening of the tubular and glomerular basement membranes; glomerulosclerosis; interstitial fibrosis; and variable infiltrates of mononuclear inflammatory cells within the interstitium. Minimal CPN affected less than 10% of the renal parenchyma, and consisted of focal to multifocal regenerative renal tubules surrounded by a thickened basement membrane. These regenerative tubules were small and lined by cuboidal basophilic epithelial cells. Mild CPN affected approximately 10% to 39% of the renal parenchyma and consisted of multifocal clusters of regenerative renal tubules, tubules that contained protein, glomeruli with thickened basement membranes, and scattered infiltrates of predominantly lymphocytes and macrophages. Moderate CPN had similar but more severe and widespread changes including glomerular atrophy and variable interstitial fibrosis. Marked CPN was diffuse and of greater severity. Mineralization was generally of minimal to mild severity and consisted of linear deposits of lamellated mineral within the lumen or epithelial cells of the collecting tubules of the renal papilla. Photomicrographs demonstrating marked CPN and linear mineralization in male rats are available elsewhere (NTP, 2007).

There were exposure concentration-related increases in minimal to mild transitional epithelial hyperplasia in the renal pelvis of male rats, which were significant at 900 and 1800 ppm (Table 1). Transitional epithelial hyperplasia consisted of focal proliferation of the transitional epithelium lining the renal pelvis; the affected epithelium appeared thickened and often formed papillary projections into the urinary space. Photomicrographs demonstrating this lesion in male rats are available elsewhere (NTP, 2007).

In the single section analysis (standard evaluation) of the kidney in males (Table 1), increases in renal tubule hyperplasia were significant at 450 and 1800 ppm, and the severities in these groups were elevated. There were also slight increases in renal tubule adenoma, carcinoma, and adenoma or carcinoma (combined). There were significant positive trends for adenomas and adenomas or carcinomas (combined). Although not statistically significant, the incidences of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) in the 900 and 1800 ppm groups and renal tubule carcinoma in the 1800 ppm group exceeded the historical ranges for chamber controls from inhalation studies fed NTP-2000 diet.

In the extended evaluation of the kidneys (Table 1), additional renal tubule hyperplasias were observed in all exposed groups such that in the combined single and step section analysis, the incidences of hyperplasia in exposed groups were significantly greater than that of the chamber controls. Additional renal tubule adenomas were observed in all groups including the chamber controls. No additional renal tubule carcinomas were observed. In the combined single and step section analysis of renal neoplasms, there were significant positive trends for renal

adenoma and adenoma or carcinoma (combined) and the incidences of these lesions were significantly increased at 1800 ppm.

Hyperplasia occurred as single or multiple expanded cortical tubules composed of increased numbers of tubular epithelial cells arranged in multiple layers that partially or completely filled the tubule. Renal tubule adenomas were discrete, highly cellular, proliferative lesions that were larger than focal hyperplasias (generally greater than the combined diameter of five normal-sized renal tubules). Adenomas tended to have a more complex structure than hyperplasias and were characterized by closely packed tubules and solid nests composed of cells with large vesicular nuclei and abundant pale eosinophilic cytoplasm which sometimes contained clear vacuoles. Renal tubule carcinomas were highly cellular, expansive and invasive masses composed of large basophilic to amphophilic cells that formed large multilayered tubular structures, solid nests, and sheets. Photomicrographs demonstrating renal tubule hyperplasia, renal tubule adenoma, and renal tubule carcinoma in male rats are available elsewhere (NTP, 2007).

Renal mesenchymal tumors occurred in two female rats in the 1800 ppm group (Table 2). Both neoplasms observed in this study were single, small to medium-sized masses with poorly defined margins and were composed of sheets of mature mesenchymal (spindle) cells that infiltrated the inner cortex, medulla, and renal pelvis encircling and sequestering glomeruli, tubules, and collecting ducts.

Lesions at sites other than the kidney were also observed in males. Mononuclear cell leukemia (0 ppm, 25/50; 450 ppm, 26/50; 900 ppm, 32/50; 1800 ppm, 35/50) increased with a significant positive trend and at 1800 ppm, the increase was significant and exceeded the historical ranges for chamber controls from inhalation studies fed NTP-2000 diet (188/399, 47% ± 10%; range 32%–66%). Adrenal gland medulla hyperplasia was also significantly increased at 1800 ppm (0 ppm, 13/50; 450 ppm, 18/48; 900 ppm, 18/50; 1800 ppm, 24/50). There were also exposure-related increases in benign or malignant pheochromocytoma (combined) of the adrenal gland in male rats (0 ppm, 8/50; 450 ppm, 9/48; 900 ppm, 11/50; 1800 ppm, 14/50). However, these increases were not significant and were within the historical ranges for chamber controls from inhalation studies fed NTP-2000 diet (69/398, 17% ± 7%; range 10%–28%), although the incidence in the 1800 ppm group was the upper limit of the historical range.

3.2. 2-year study in B6C3F1 mice

Survival of male (0 ppm, 40/50; 450 ppm, 42/50; 900 ppm, 35/50; 1800 ppm, 37/50) and female (0 ppm, 35/50; 450 ppm, 37/49; 900 ppm, 39/50; 1800 ppm, 38/50) mice was similar to that of the chamber controls. Mean body weights of male mice were generally similar to those of the chamber controls throughout the study. After week 17, body weights of 1800 ppm females were 9–16% less than those of the chamber controls. No clinical findings related to exposure to MIBK were observed.

Eosinophilic foci in the liver were increased in all exposed groups of female mice, and the differences from the chamber controls were significant in the 450 and 1800 ppm groups; this lesion was not significantly increased in exposed male mice. There were significant positive trends for hepatocellular adenomas in both males and females and the incidences in males (all exposed groups) and females (900 and 1800 ppm) were at the upper limit or exceeded the historical control ranges; these increases were significant at 1800 ppm in both males and females (Table 3). Multiple hepatocellular adenomas were also increased in both males and females; there were significant positive trends in both sexes and significant increases at 900 ppm (females) and 1800 ppm (males and females) (Table 3). Hepatocellular carcinoma was elevated in females at 1800 ppm (Table 3); although not statistically significant, this increase exceeded the historical control range. There were significant positive trends for hepatocellular

adenomas or carcinomas (combined) and the incidences exceeded the historical control ranges in males (1800 ppm) and females (900 ppm or 1800 ppm); these increases were significant at 1800 ppm in both males and females (Table 3).

The histologic appearance of the hepatocellular proliferative lesions was consistent with those that develop spontaneously in control mice. Eosinophilic foci consisted of enlarged hepatocytes with ground-glass appearing cytoplasm; larger foci sometimes caused slight compression of the adjacent parenchyma. Hepatocellular adenomas were discrete, variably sized, circumscribed masses with variable compression of the adjacent normal parenchyma. Adenomas were composed of well-differentiated hepatocytes that had mild cellular pleomorphism. Hepatic cords were irregular and abruptly impacted the adjacent parenchyma at right angles. Hepatocellular carcinomas were expansive, invasive masses characterized by irregular borders and a trabecular pattern consisting of hepatic cords greater than three to four cells wide; the neoplastic hepatocytes varied from well-differentiated to markedly atypical with enlarged hyperchromatic atypical nuclei and one or more prominent nuclei.

4. Discussion

The short-term inhalation toxicity of MIBK has been previously characterized; however, no studies investigating the chronic toxicity and carcinogenicity of MIBK have been reported. The objective of the present studies was to characterize the toxicity and carcinogenicity of MIBK in F344N rats and B6C3F1 mice following exposure to 0, 450, 900 or 1800 ppm for two years by inhalation. The primary findings of these studies are presented in this report; a more detailed report can be found in NTP TR 538 (NTP, 2007).

Exposure concentrations for the present 2-year studies in rats and mice were selected following the review of previously conducted inhalation toxicity studies (Phillips *et al.*, 1987). Based on the toxicity data from these studies, it was anticipated that 2000 ppm would likely exceed the maximum tolerated dose for a 2-year exposure period. Therefore, 1800 ppm was selected as the highest exposure concentration for the present studies in both rats and mice. The lower exposure concentrations were spaced by half to examine the dose-response of toxic or carcinogenic effects of MIBK.

In the present study, exposure of male rats to MIBK resulted in decreased survival at 1800 ppm and decreased body weight at 900 and 1800 ppm. These effects were not observed in female rats, or male mice. There were decreased body weights in female mice at 1800 ppm. The major target organ sites of toxicity and carcinogenicity were the kidney in rats and the liver in mice.

CPN was observed in almost all male rats including the chamber controls. However, there were treatment related significant increases in both the incidence (1800 ppm) and severity (all exposed groups). Although CPN is one of the most commonly recognized spontaneous lesions in the rat (Seely *et al.*, 2002), this condition can be exacerbated by chemical exposure, leading to increased incidences and average severities (Lock and Hard, 2004). CPN as a syndrome is more prevalent and severe in male rats. However, there were also significant increases in both the incidence (all exposed groups) and severity (1800 ppm) of CPN in females. Hyperplasia of the transitional epithelium lining the renal pelvis was increased in exposed males, and the increases were significant at 900 and 1800 ppm. Such hyperplasia frequently accompanies severe CPN (Montgomery and Seely, 1990), and the increased incidences in the current study may reflect the exacerbated CPN. Linear mineralization of the epithelium of collecting ducts in the renal papilla, which frequently accompanies CPN, was significantly increased in all exposed groups of males.

Because renal tubule hyperplasia, adenoma, and carcinoma represent a continuum in the progression of proliferative lesions in the kidney and because significant increases in

hyperplasia and marginal increases in adenoma, carcinoma, and adenoma or carcinoma (combined) were identified by the standard single-section evaluation of the kidney in males, step sections were evaluated in an extended evaluation. Additional hyperplasias in all exposed groups and adenomas in all groups were identified in the step section analysis; additional carcinomas were not identified. When the results of the single and step sections were combined, there were significant increases in renal tubule hyperplasia at all exposure concentrations and in adenoma and adenoma or carcinoma (combined) at 1800 ppm. These lesions were not observed in females; however, renal mesenchymal tumors were identified in two female rats in the 1800 ppm exposure group. This very rare tumor has not been observed in male or female control animals fed NTP 2000 diet in NTP studies (all routes of administration). In treated F344/N rats fed NTP 2000 diet, mesenchymal tumors were identified in only one male and three female rats in three 2-year studies including the current study. The occurrence of this neoplasm in only two female rats makes the relationship to MIBK exposure uncertain.

The spectrum of kidney lesions described in the literature after short-term exposures to MIBK (Phillips, et al., 1987; Nemec, et al., 2004) supports the observation that the kidney is the primary target in rats. In the 3-month study of MIBK (Phillips, et al. 1987), there were dose-related increases in the severity of hyaline droplet formation and regeneration of PCT cells in male rats, although the highest dose tested in the 13-week study was 1,000 ppm. In the present study, there were increases in the incidence and severity of CPN, linear mineralization in the renal papilla, and renal tubule hyperplasia in male rats. Minimal hyaline droplet accumulation was observed in two 900 ppm and two 1800 ppm male rats that died relatively early in the study; hyaline droplet formation diminishes with age and is unlikely to be detectable in rats at the end of a 2-year study. These lesions are suggestive of α2u-globulin nephropathy, a syndrome specific to male rats.

α2u-Globulin neophropathy is thought to be a possible mechanism of xenobiotic-induced renal carcinogenesis (Montgomery and Seely, 1990); the pathophysiology, diagnostic characteristics, and relevance of α2u-globulin neophropathy in human risk assessment have been extensively discussed in the literature (Swenberg et al., 1989; Borghoff et al., 1990; USEPA, 1991; IARC, 1999). The proposed sequence of events involved in the induction of α2u-globulin neophropathy includes binding of a chemical to the male rat specific protein α2u-globulin, accumulation of the complex in phagolysosomes of renal proximal tubule cells (hyaline droplets), and a cycle of cytotoxicity, apoptotic death and compensatory cell proliferation, that if chronic, may lead to the promotion of neoplasia. Alternatively, it has been proposed that α2u-globulin may serve as a vector to increase the delivery of a toxicant or protoxicant to proximal tubule cells, leading to an elevated chemical concentration in the male rat kidney (Melnick, 1992). Further studies, would be necessary to more definitively identify MIBK as an inducer of α2u-globulin nephropathy. These studies would include a thorough characterization of renal histopathology, characterization of the binding affinity of MIBK with α2u-globulin, and quantitation of cell proliferation, at exposure concentrations of 2000 ppm or greater, with inclusion of doses used in the present study.

Doi and coworkers recently reviewed selected NTP studies of compounds that induced α2u-globulin nephropathy; studies examined in this review were d-limonene, decalin, Stoddard Solvent IIC, and propylene glycol mono-t-butyl ether (Doi, et al., 2007). The authors identified lesions generally observed in prechronic studies, which included α2u-globulin accumulation, cell proliferation, hyaline droplet accumulation, tubular regeneration, and granular cast formation, and lesions generally observed in chronic studies, which included CPN, mineralization, and renal tubule hyperplasia. The authors concluded that exacerbated CPN and linear mineralization in the renal papilla, indicators of sustained damage, were the best predictors of the tumor outcome, but that the pathologic presentation of α2u-globulin

nephropathy was variable and that no one event or set of pathologic events clearly predicted tumor response.

Increases in cell turnover, associated with CPN arising from α2u-globulin dependent or independent mechanisms, are recognized kidney tumor risk factors (Hard, 1998; Hard et al., 1997; Swenberg et al., 1989). There does not appear to be a counterpart of CPN or α2u-glubulin nephropathy in humans. In an evaluation of the renal histopathological changes occurring in ethylbenze-treated rats from NTP-sponsored studies (NTP, 1999), Hard concluded that tumor development was due to chemically-induced exacerbated CPN (Hard, 2002); these effects occurred in male and female rats. Increases in both exacerbated CPN and renal tubule tumors have been noted in several other NTP studies, including coumarin (NTP, 1993), primidone (NTP, 2000) and benzophenone (NTP, 2006). Thus, the increase in the severity of the CPN in the present study, whether dependent on or independent of α2u-globulin, likely contributed to the increase in renal tubule tumors. α2u-Globulin dependent mechanisms did not appear to be involved in the renal effects observed in any of these studies. Conversely, although there were increases both the incidence and severity of CPN in female rats, the association between MIBK exposure and renal tumor induction was uncertain, and there was no evidence of renal tubule tumor induction, in females. The exacerbated CPN in females was not due to α2u-globulin nephropathy, as female rats produce little if any hepatic α2u-globulin and thus do not develop α2u-globulin nephropathy (MacInnes et al., 1986; Chatterjee et al., 1989; Lehman-McKeeman and Caudill, 1992).

There were findings in tissues other than the kidney in male rats. Although there was a significant increase in mononuclear cell leukemia at 1800 ppm, the strength of the response made the finding uncertain. There were increases in adrenal medulla hyperplasia, which were significant at 1800 ppm. There was also a positive trend for increases in benign or malignant pheochromocytomas (combined); however increases were neither statistically significant nor exceeded the historical control range. Nemec *et al.* (2004) reported significant changes in adrenal gland weights of female Sprague-Dawley rats exposed to 2,000 ppm MIBK for 70 days, which were the F0 generation of a two generation reproductive study.

The liver was the sole target tissue in male and female B6C3F1 mice in the present study. Eosinophilic foci were increased in females at 450 and 1800 ppm; incidences in exposed males were similar to controls. Hepatic foci are more frequently observed in mice treated with hepatocarcinogens than untreated controls, and although there is evidence linking these lesions to the development of hepatocellular neoplasms, their exact role in hepatocarcinogenesis is still uncertain (Harada *et al*., 1999). In general, these lesions precede the development of hepatic neoplasms and may increase in incidence and multiplicity with time and administration of liver carcinogens. However, while some foci progress to neoplasia, others regress when the inciting carcinogenic stimulus is removed.

Induction of hepatocellular neoplasms following MIBK treatment was similar between male and female mice. In both sexes, increases in hepatocellular adenomas and adenoma or carcinomas (combined) were significant and exceeded historical control ranges at 1800 ppm. There was also increased multiplicity of hepatocellular adenomas at 900 ppm in females and 1800 ppm in males and females. There were no significant increases in carcinomas in either sex, although the incidence was elevated in females at 1800 ppm. Although hepatocellular adenoma is the most frequent spontaneous liver neoplasm in B6C3F1 mice, the combination of increased hepatocellular neoplasms and increased multiplicity in exposed males and females supports the carcinogenic activity of MIBK in the liver. The similarities in exposure-responses between males and females suggest that similar mechanisms are responsible for the induction of hepatocellular tumors in male and female mice, or at least are independent of the sex of the animal.

Findings reported in prechronic studies provide support for the liver as the target organ of MIBK-related toxicity in mice. Effects on the liver in prechronic studies included increased liver weights and centrilobular hypertrophy (Phillips *et al*., 1987, Nemec *et al*. 2004). Although the significance of hepatocellular hypertrophy in the liver carcinogenic response is not completely understood, it was identified as the best single predictor of liver cancer in a recent survey of 111 NTP studies over a 10-year period (Allen *et al.*, 2004). Although it has been considered an adaptive response to excessive metabolic load (Schulte-Hermann, 1974), hepatocellular hypertrophy was not observed during histologic evaluation of mice in the present study. Induction of P450 enzymes by MIBK may have contributed to the observed lesions in the liver in mice.

In conclusion, following two-year inhalation exposure, the targets of toxicity and carcinogenicity of MIBK were the kidney in rats and the liver in mice. In male rats, the observed CPN and increase in renal tubular tumors following MIBK exposure may have resulted from an α2u-globulin-related mechanism; however, since exacerbated CPN was also observed in female rats, additional mechanisms were likely involved. Increases in mononuclear cell leukemias in male rats and the occurrence of two renal mesenchymal tumors in female rats were uncertain findings. Increases in hepatocellular tumors and tumor multiplicity were similar between male and female mice and MIBK was considered to be a hepatocarcinogen in both sexes.

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Table 1

Incidences of neoplasms and nonneoplastic lesions of the kidney in male F344/N rats exposed to methyl isobutyl ketone by inhalation for two years.

	0 ppm	450 ppm	900 ppm	1800 ppm
Number Examined Microscopically	50	50	50	50
Nephropathy				
Single Sections	42[a] (2.0)[b]	45 (2.6)▲	47 (2.4)▲	50[*] (3.1▲
Papilla Mineralization				
Single Sections	1 (1.0)	6[*] (1.2)	22[**] (1.6)	29[**] (1.5)
Pelvis Transitional Epithelium Hyperplasia				
Single Sections	1 (1.0)	5 (1.8)	6[*] (1.2)	19[**] (1.4)
Renal Tubule Hyperplasia				
Single Sections	1 (2.0)	11[**] (3.2)	3 (2.0)	18[**] (2.7)
Step Sections	0	3 (2.0)	4 (2.0)	6[*] (2.3)
Single and Step Sections (Combined)	1 (2.0)	14[*] (2.9)	7[*] (2.0)	21[**] (2.5)
Renal Tubule Adenoma[c]				
Single Sections	0[*]	0	2 (4.7)[d]	3 (7.3)
Step Sections	2 (4.5)[*]	3 (7.2)	1 (2.4)	7 (16.8)
Single and Step Sections (Combined)	2 (4.5)[**]	3(7.2)	3 (7.1)	10 (24.0)[**]
Renal Tubule Carcinoma[e]				
Single Sections	0	1 (2.4)	0	2 (4.8)
Step Sections	0	0	0	0
Single and Step Sections (Combined)	0	1 (2.4)	0	2 (4.8)
Renal Tubule Adenoma or Carcinoma[f]				
Single Sections	0[*]	1 (2.4)	2 (4.7)	4 (9.7)
Step Sections	2 (4.5)[*]	3 (7.2)	1 (2.4)	7 (16.8)
Single and Step Sections (Combined)	2 (4.5)[**]	4 (9.5)	3 (7.1)	11 (26.4)[**]

[a] lesion incidence;

[b] average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked;

[c] Historical incidence for 2-year inhalation studies with chamber controls given NTP-2000 diet (mean ± standard deviation): 3/399 (0.8% ± 1.0%); range, 0%–2%;

[d] survival adjusted incidence (%);

[e] Historical incidence: 1/399 (0.3% ± 0.7%); range, 0%–2%;

[f] Historical incidence: 4/399 (1.0% ± 1.1%); range, 0%–2%;

▲ Increased lesion severity by Mann Whitney U test;

[*] P≤0.05 by the poly-3 test (for trend if assigned to control);

[**] P≤0.01

Table 2

Incidence of neoplasms and nonneoplastic lesions of the kidney in female F344/N rats exposed to methyl isobutyl ketone by inhalation for two years.

	0 ppm	450 ppm	900 ppm	1800 ppm
Number Examined Microscopically	50	50	50	50
Nephropathy	19[a] (1.4)[b]	35[**] (1.5)	38[**] (1.5)	44[**] (1.9)▲
Mesenchymal tumor malignant[c]	0[*]	0	0	4 (4.6)[d]

[a] lesion incidence;

[b] average severity grade of lesion in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked;

[c] Historical incidence for 2-year inhalation studies with chamber controls given NTP-2000 diet: 0/396;

[d] survival adjusted incidence (%);

[*] $P \leq 0.05$ by the poly-3 test (for trend if assigned to control);

[**] $P \leq 0.01$

Table 3

Incidence of neoplasms and nonneoplastic lesions of the liver in male and female B6C3F1 mice exposed to methyl isobutyl ketone by inhalation for two years.

	0 ppm	450 ppm	900 ppm	1800 ppm
Number Examined Microscopically	50	50	50	50
Eosinophilic Foci				
Males	3[a]	4	5	8
Females	4	11**	10	14**
Hepatocellular Adenoma				
Males[b]	17 (36.4)[c]**	25 (52.0)	23 (51.9)	34 (71.9)**
Females[d]	13 (28.8)*	15 (32.4)	20 (43.1)	23 (49.3)*
Multiple Adenoma				
Males	6 (12.9)*	11 (22.9)	12 (27.2)	15 (30.9)*
Females	2 (4.5)**	5 (10.5)	8 (17.4)*	14 (29.9)**
Hepatocellular Carcinoma				
Males[e]	12 (25.0)	12 (24.4)	10 (22.8)	9 (19.2)
Females[f]	6 (13.1)	5 (10.8)	6 (12.9)	11 (23.5)
Hepatocellular Adenoma or Carcinoma				
Males[g]	27 (56.1)*	34 (68.3)	28 (61.6)	37 (77.3)*
Females[h]	17 (37.1)*	17 (36.4)	22 (46.9)	27 (57.6)*

[a] lesion incidence;

[b] Historical incidence for 2-year inhalation studies with chamber controls given NTP-2000 diet (mean ± standard deviation):134/350 (38.3% ± 6.3%); range, 30%–46%;

[c] survival adjusted incidence (%);

[d] Historical incidence: 78/347 (22.5% ± 8.1%); range, 12%–35%;

[e] Historical incidence: 85/350 (24.3% ± 4.8%); range, 18%–32%;

[f] Historical incidence: 37/347 (10.7% ± 1.8%); range, 8%–12%;

[g] Historical incidence: 196/350 (56.0% ± 6.2%); range, 50%–68%;

[h] Historical incidence: 108/347 (31.1% ± 6.8%); range, 22%–39%;

* $P \leq 0.05$ by the poly-3 test (for trend if assigned to control tumor incidence);

** $P \leq 0.01$